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# New membrane assembly in IgE receptor-mediated exocytosis

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## Summary

The presence of excess membrane has been observed in the secretory granules of mast cells activated via the physiological mechanism of IgE receptor-mediated exocytosis. This excess membrane is the result of a *de novo* assembly from phospholipid, cholesterol, and other membrane components stored in the matrix of the quiescent granule. Following receptor stimulation, membrane bilayer structures of varying size and shape can be seen in the subperigranular membrane space where the perigranular membrane has lifted away from the granule matrix. Vesicles as small as 25 nm in outer diameter are frequently found beneath the perigranular membrane at the site of granule fusion. Membrane in the form of elongated vesicles, tubes, or sheets has also been observed. The wide variation in size and shape of the newly assembled membrane may reflect the spontaneity of the entropy-driven membrane generation process and the fluid characteristic of the biological membrane in general. Fusion of the newly assembled membrane with the perigranular membrane enables the activated granule to enlarge. This rapid expansion process of the perigranular membrane may be the principal mechanism by which an activated granule can achieve contact with the plasma membrane in order to generate pore formation. The fact that new membrane assembly also occurs in the IgE receptor-mediated granule exocytosis, supports our observation that *de novo* membrane generation is an inherent step in the mechanism of mast cell granule exocytosis. Whether new membrane assembly is a common step in the mechanism of secretory granule exocytosis in general, must await careful reinvestigation of other secretory systems.

## Introduction

Perigranular membrane lifting and granule enlargement are early morphological changes associated with mast cell exocytosis and histamine release (Bloom & Haegermark, 1965; Bloom & Chakravarty, 1970; Rohlich *et al.*, 1971; Uvnas, 1982). In agreement with the above observation, we have previously proposed a mechanism by which this rapid membrane expansion could be accomplished (Chock & Chock, 1985; Chock & Schmauder-Chock, 1985). The conclusion, which led to our proposing that a *de novo* membrane assembly is an obligatory step in the mechanism of secretory granule exocytosis, was based on the following findings:

(1) Quiescent mast cell granules contain no excess membrane within them, but following stimulation to secrete histamine, membrane vesicles can be observed in association with activated granules.

(2) Purified granules, after their perigranular mem-

branes have been removed, can still generate new bilayer membrane.

(3) New membrane vesicles have been seen fusing with the enlarging perigranular membrane in mast cells activated with detergent (Chock & Schmauder-Chock, 1985).

(4) The activated granule has been observed to triple in its perigranular membrane surface area prior to fusion with the plasma membrane (Schmauder-Chock & Chock, 1987a).

(5) A non-bilayer phospholipid pool, capable of sustaining a trebling of the granule surface area, has been localized in the quiescent mast cell granule (Chock & Schmauder-Chock, 1987, 1989). This phospholipid pool also serves as the source of arachidonic acid needed for the production of prostaglandins and other eicosanoids during exocytosis (Chock & Schmauder-Chock, 1987, 1988; Schmauder-Chock & Chock, 1989).

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In order to find out if *de novo* membrane assembly also occurs in the normal physiological mechanism of histamine release, we have now examined the rapid granule changes following an antigen challenge of presensitized mast cells.

Physiologically, the production of immunoglobulin E (IgE) antibodies in response to antigen (e.g. allergens) exposure is the cause of immediate hypersensitivity. The binding of these resulting antibodies to the mast cell surface IgE receptors renders the mast cell sensitive to the antigen (Ishizaka *et al.*, 1970). Challenge of presensitized mast cells with their specific antigen will trigger granule exocytosis and result in the release of histamine. By presensitizing rat peritoneal mast cells with a monoclonal IgE antibody against DNP, and then challenging them with a multivalent DNP antigen, we also found the presence of excess membrane within the activated granules. Since this excess membrane and also that which has been shown in the detergent-activated mast cell are the result of a new assembly, we concluded that *de novo* membrane generation must also occur in the normal IgE receptor-mediated mast cell secretory granule exocytosis. This, in turn, suggests that new membrane assembly may be an inherent step in the mechanism of secretory granule exocytosis.

### Materials and methods

Male Sprague-Dawley rats (350–500 g) were either obtained from the NIH animal facility or from NIH animal contract suppliers. Peritoneal lavages were performed after the animals had been killed by carbon dioxide inhalation. A 20 ml cold Hank's balanced salt solution (HBSS, Gibco) containing 0.1% bovine serum albumin was injected into the peritoneal cavity and drained through an incision several minutes later. A fraction, enriched in mast cells, was obtained from the 30 xg, 10 min low speed centrifugation of the combined peritoneal lavages. Passive sensitization against 2,4-dinitrophenol (DNP) epitopes was based primarily on the original procedure of Ishizaka *et al.* (1985). After one wash with calcium-free HBSS, the cell suspension was incubated for one hour at 20°C in the presence of 100 µg ml<sup>-1</sup> anti-DNP monoclonal IgE antibody to saturate the surface IgE receptors. Unbound antibodies were removed by three subsequent washings of the 35 xg 10 min centrifugation pellets: twice with calcium-free HBSS and once with normal HBSS. After resuspension in HBSS, the sensitized mast cells were challenged with the multivalent antigen DNP cross-linked to human serum albumin (DNP<sub>21</sub>-HSA). After a 45 sec incubation in the presence of 0.6 µg ml<sup>-1</sup> DNP<sub>21</sub>-HSA at 20°C, the reaction was terminated by adding an equal volume solution of 4% glutaraldehyde, 4 mM MgCl<sub>2</sub> in 100 mM sodium cacodylate, pH 7. After 30 min at room temperature, the cells were quickly washed twice with the fixative buffer containing no glutaraldehyde, using a Beckman desktop centrifuge at 1 min each spin. The washed cells were postfixed in 1% osmium tetroxide for 30 min, dehydrated in an acetone series and embedded in Epon-812. Thin

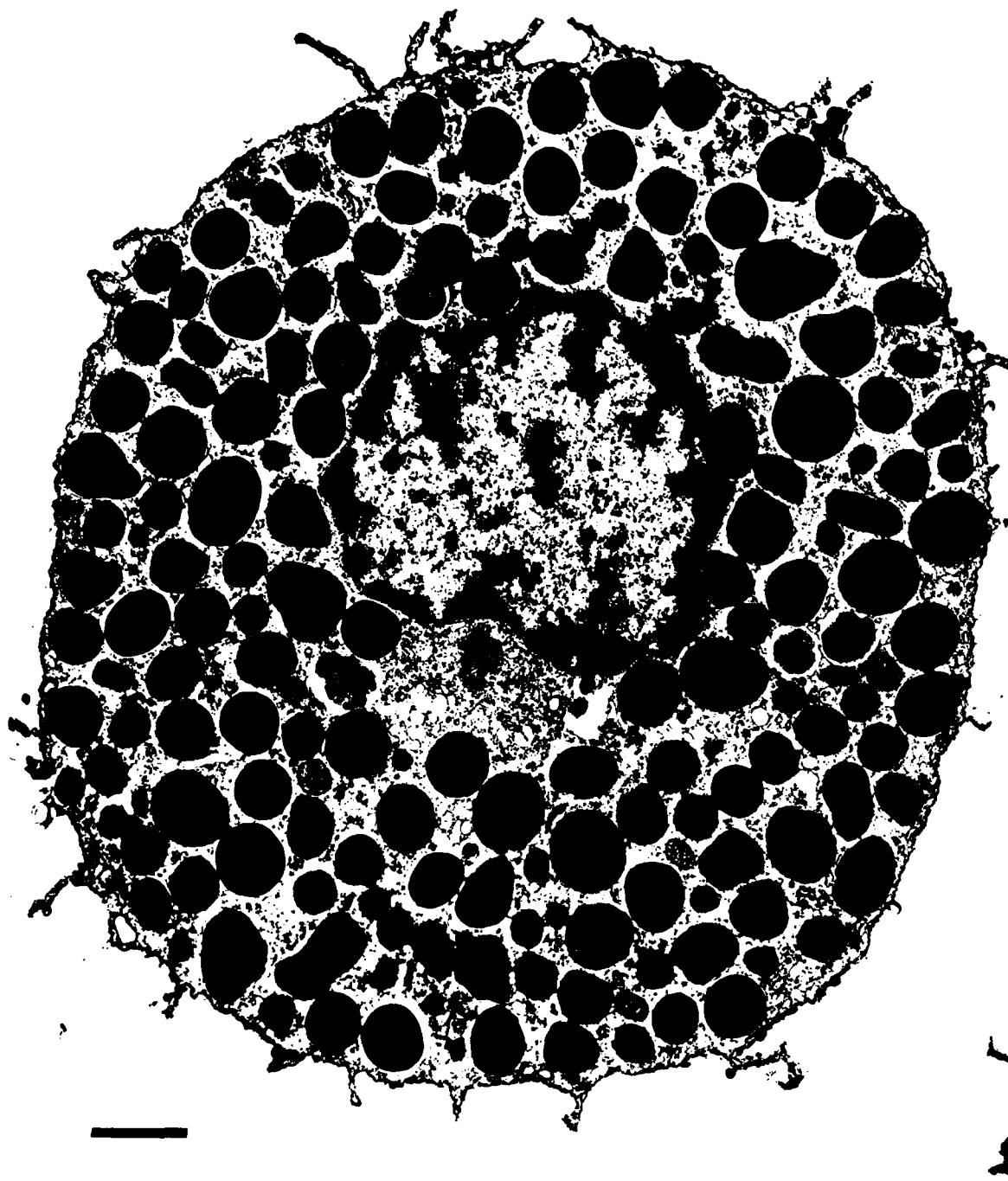
sections were also stained with Reynold's lead citrate and uranyl acetate before examination with a Philips 400 electron microscope.

### Results

Rat connective tissue mast cells, isolated by peritoneal lavage, contain a large number of secretory granules (Fig. 1). The quiescent granule is surrounded by a taut perigranular membrane which is often indiscernible due to an overwhelming electron density of the granule matrix. Rapid freezing and freeze-fracture of unstimulated mast cells also reveal the presence of very smooth and well-rounded perigranular membrane (Chandler & Heuser, 1980). Apart from the many mediators of anaphylaxis (Metcalf *et al.*, 1981; Uvnas, 1982), we recently showed that the mast cell granule also contains a large amount of phospholipid, cholesterol, calcium, phospholipase A<sub>2</sub>, cyclooxygenase and other enzymes of the arachidonic acid cascade (Chock *et al.*, 1982, 1984; Albers *et al.*, 1985; Chock & Schmauder-Chock, 1987, 1988, 1989; Schmauder-Chock & Chock, 1989). The quiescent granule is very condensed and has a specific gravity of about 1.2 (Kruger *et al.*, 1980). It contains very little or no water. The absence of an aqueous environment in the granule matrix also explains why the matrix-bound phospholipid can exist in a non-bilayer form (Chock & Schmauder-Chock, 1989). The absence of water also prevents the granule enzymes from becoming active before the granule is activated.

A process of *de novo* membrane assembly has been shown to occur (Chock & Chock, 1985; Chock & Schmauder-Chock, 1985) in mast cell granules activated by a detergent. Assembly of the new membrane must have been triggered by an influx of cellular water into the activated granule, which causes a spontaneous sequestration and coalescence of granule-stored phospholipid into membrane vesicles. Fusion of these newly assembled vesicles into the perigranular membrane explains how the perigranular membrane of the activated granule can rapidly enlarge prior to fusion with the plasma membrane (Chock & Schmauder-Chock, 1985, 1989). For the IgE receptor-mediated granule exocytosis, evidence which suggests the occurrence of a similar process can also be observed (Fig. 2).

In Fig. 3, views A–G represent high magnification of areas labelled A–G respectively in Fig. 2. Evidence of excess membrane, frequently in the form of vesicles, is found within granules in the early stages of activation when the granule matrices are still quite electron dense (Fig. 3B, E, F, and G), as well as in granules which have decondensed and become reticular in appearance (Fig. 3A, C and D). Many vesicles are seen to contain matrix material. In Fig. 3A, the matrix material contained within the enclosing membrane.

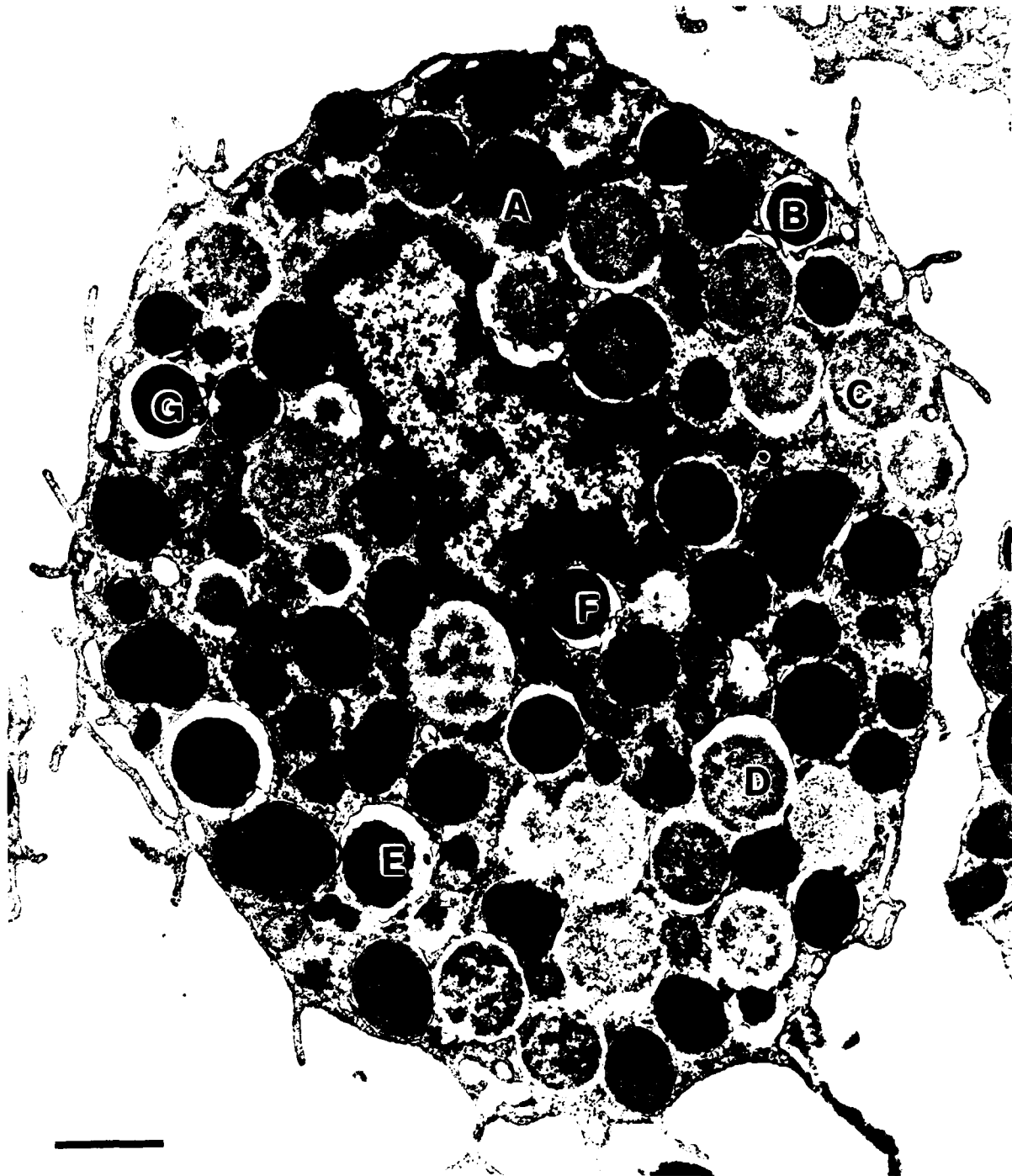


**Fig. 1.** Unstimulated mast cell. Rat peritoneal mast cells isolated by peritoneal lavage, showing amorphous, electron-dense, membrane-delimited secretory granules. The bar at the lower left corner represents 1  $\mu$ m.

('large vesicle') might have spontaneously assembled into small vesicles in response to a water influx into the enclosing membrane. Some of the enclosed vesicles are as small as 23 nm in outer diameter. Vesicles of around 25 nm in diameter are frequently seen in decondensing granules.

Figure 4A shows a section of a cell in its early stage of activation; most of its granules are still electron dense.

However, many of the perigranular membranes have already lifted away from their matrices. Close observation of selected areas, labelled b-h, can be seen under high magnification (Fig. 4 B-H respectively) to contain newly assembled membrane vesicles (arrows). Of particular interest is the chain of granules in a line from the plasma membrane to the nucleus (labelled h, g, f and e in Fig. 4A). Under high magnification, their

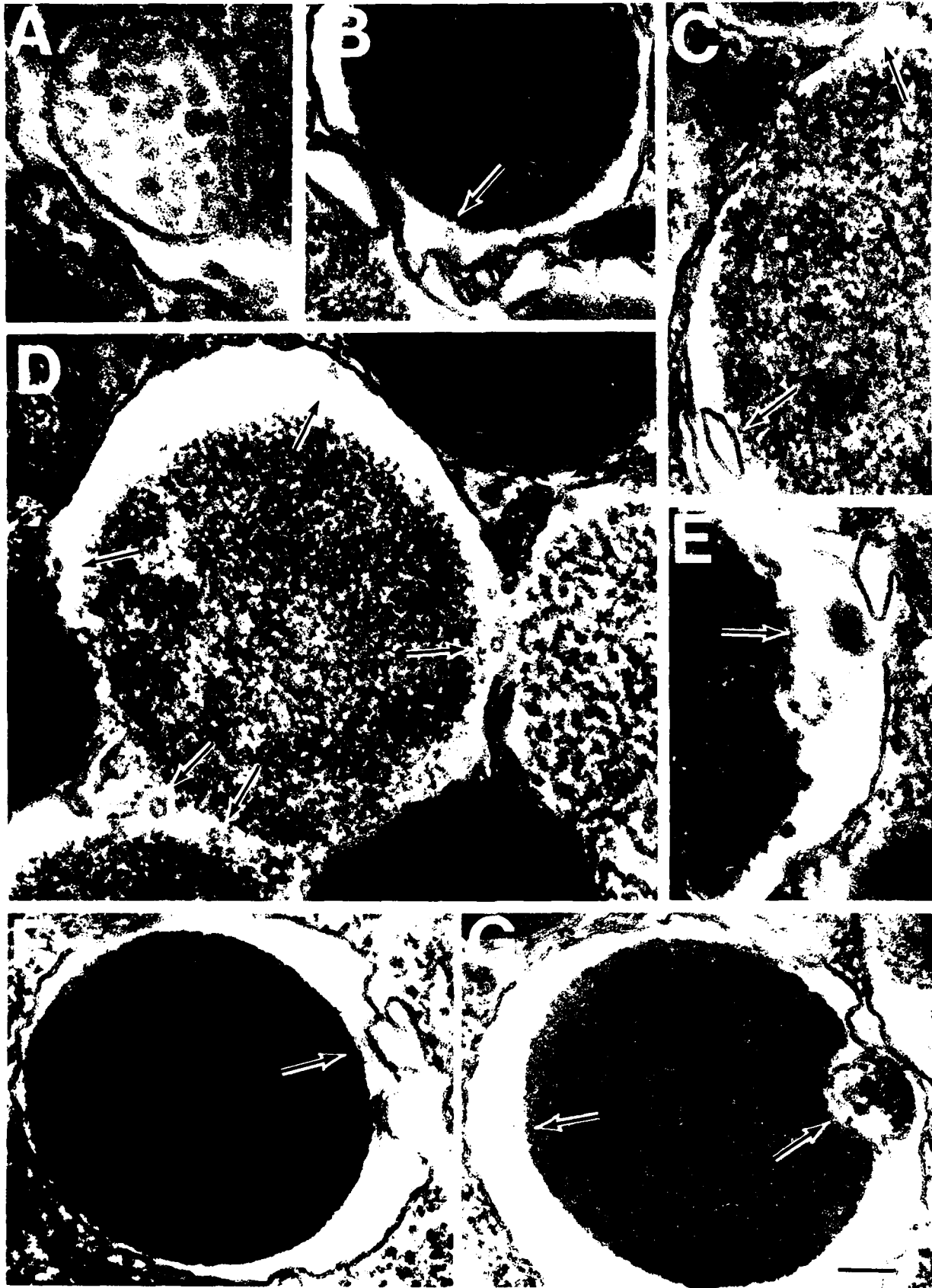


**Fig. 2.** IgE receptor-mediated mast cell activation. Presensitized mast cells 45 sec after specific antigen challenge show gross transformation of their secretory granules. The areas marked A-G correspond to areas shown at high magnification in Fig. 3. Perigranular membrane lifting and granule matrix dispersion are apparent in several granules throughout the stimulated cell. The bar at the lower left corner represents 1  $\mu$ m.

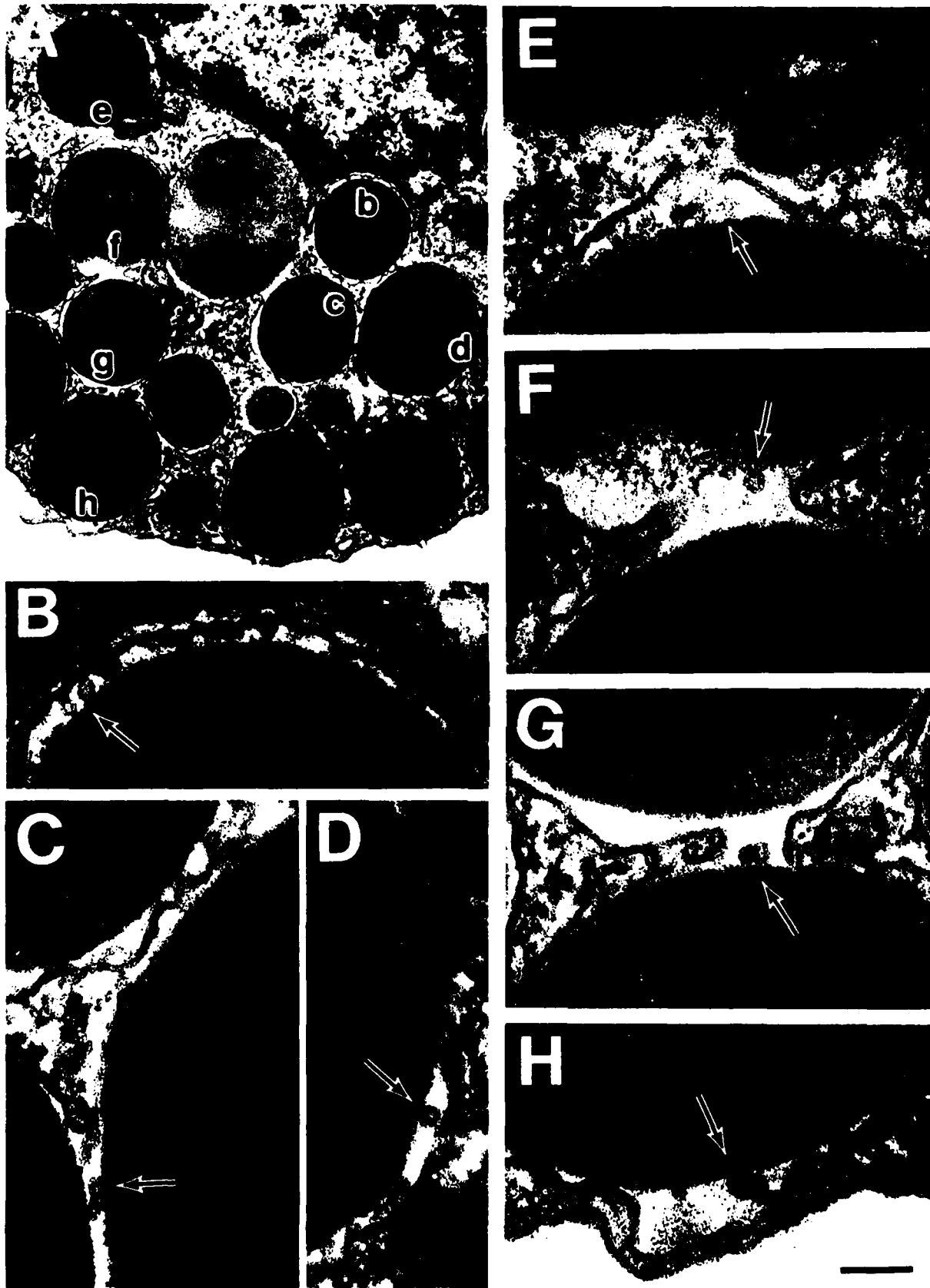
fusion with each other becomes apparent (Fig. 4 E-H), and the presence of new membrane vesicles (arrows) at the sites of fusion may suggest their fusogenic role in this case. If the fusion of these granules with each other occurs before their fusion with the plasma membrane (pore formation), this would result in the

formation of a membrane-lined common vacuole. Subsequent fusion of this vacuole with the plasma membrane will result in the formation of a deep channel open to the outside and simultaneous exteriorization of many granule matrices.

Figure 5 shows two different activated granules in



**Fig. 3.** High magnification of activated granules reveals the presense of new membranes of varying size and shape at the space between the lifted perigranular membrane and granule matrix. In (A), small vesicles, some as small as 23–25 nm in diameter, appear to have formed from matrix components enclosed within the delimiting vesicles in response to a water influx. In (B–G) membranes of varying size and shape (arrows) are seen within the activated granules. The bar at the lower right corner represents 0.1  $\mu\text{m}$  when applied to the images in (B) (C) (E) (F) and (G). The same bar, when applied to the images in (A) and (E) is equivalent to 0.06 and 0.12  $\mu\text{m}$  respectively.



**Fig. 4.** New membrane vesicles and their presence at the sites of granule fusion during the formation of a membranelined common vacuole. A low magnification image of an antigen-challenged mast cell, showing granules at an early stage of activation A. Areas designated as b-h in (A) are shown under high magnification in (B-H) respectively. Small vesicles (arrows) are shown in subperigranular membrane space in (B-H). Four granules in a line, from the plasma membrane to the nucleus (h, g, f, and e in A) have fused into a connecting channel to form a common vacuole. At the sites of intergranular fusion (E, F and G), new vesicles can also be seen (arrows). The bar at the lower right corner represents  $0.49\ \mu\text{m}$  when applied to the image in A, and equals  $0.1\ \mu\text{m}$  when applied to the images in (B-H.)

the process of decondensation, at high magnification. Evidence that a new membrane assembly has already occurred is seen in the many membrane vesicles found between the lifting perigranular membrane and granule matrix of each granule. In Fig. 5A, the process of vesicle generation seems to give the impression that they are oozing out of the matrix like soap bubbles. The fact that some of the vesicles (arrowhead) can be assembled deep within the granule matrix suggests that water can penetrate deep into the granule matrix during the process of decondensation. The loosening of the matrix structure can be clearly seen as it takes on a dappled appearance (Fig. 5B). The progression of the membrane assembly process is disguised by the density of matrix which is still osmophilic. It should be noted that many of the newly formed vesicles appear to contain some matrix components. Therefore, fusion of these vesicles into the perigranular membrane would result in ejection of granule matrix materials into the cytoplasm of the activated cell (Chock & Schmauder-Chock, 1989, 1990; Schmauder-Chock & Chock, 1987b).

### Discussion

It has been established that the perigranular membrane of an activated granule can enlarge prior to matrix decondensation (Bloom *et al.*, 1965, 1967; Rohlich *et al.*, 1971; Uvnas, 1982). A trebling of the perigranular membrane surface area prior to fusion with the plasma membrane has been seen in granules of mast cells activated by the compound A23187 (Schmauder-Chock & Chock, 1987a). Extensive perigranular membrane enlargement has also been observed for the IgE receptor-mediated mast cell stimulation. An activated granule with an enlarged perigranular membrane equivalent to a surface area increase of about 40% can be seen in Fig. 5A. Since membrane bilayer cannot stretch beyond 2–3% of its original surface area (Kwok & Evans, 1981), and exocytosis is a membrane-delimited event (Palade, 1975), this tremendous enlargement of the perigranular membrane of an activated granule requires the addition of membrane from another source. Since formation of new membrane vesicles and their fusion with the activated perigranular membranes have already been observed in the detergent-activated mast cell granules (Chock & Schmauder-Chock, 1985), it can be inferred that the same process of *de novo* membrane generation must also have occurred in the IgE receptor-mediated granule exocytosis as observed here.

Several authors have described the presence of various membranous structures in association with activated granules of the mast cell (Lagunoff, 1973; Lawson *et al.*, 1977; Kruger *et al.*, 1980; Lawson, 1980). However, they attributed their observations to a fixation artefact. By using rapid freezing and freeze-

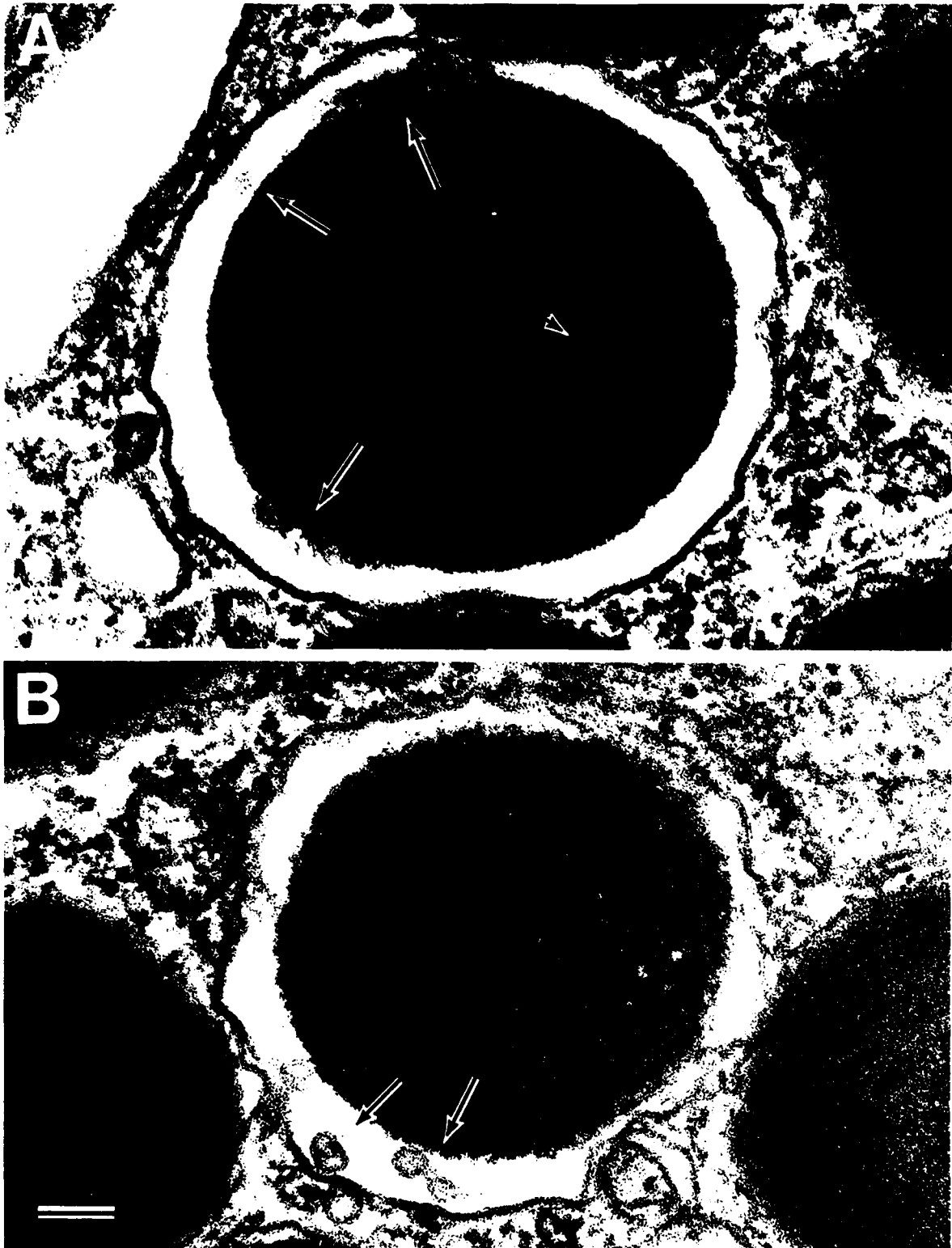
substitution, we have previously demonstrated that the mast cell granule does, indeed, have the potential to generate new membrane. This *de novo* membrane assembly process was captured in mast cell granules activated by detergent. The fact that a similar process also occurs in the IgE receptor-mediated granule exocytosis, as shown here, further supports our contention that *de novo* membrane assembly is an integral step in the mechanism of mast cell granule exocytosis. Whether such a process can be a common step in the mechanism of exocytosis in general, cannot yet be answered until more secretory systems are investigated.

However, membrane-like structures have been observed in activated platelet granules, which after stimulation, also secrete a phospholipid-derived clotting factor (Fonio, 1951; White & Krivit, 1966; Marcus *et al.*, 1969). The extensive vesiculation of eosinophil granules following activation by compound A23187, reported by Henderson and Chi (1985), can also be thought of as evidence of a new membrane assembly process similar to that occurring in the activated mast cell granule. Apart from the mast cell granule which has been shown to contain a matrix-bound phospholipid store, many other secretory granules have been suggested to contain phospholipid or lipid-like material. For example, those of the chromaffin cell (Blaschko *et al.*, 1967; Mylroie & Konig, 1971; Helle, 1973), the parotid acinar cell (Simson *et al.*, 1973), the thymus gland cells (Curtis *et al.*, 1979), the prostate gland cells (Kanwar & Kansal, 1980), and the poison gland cells of the centipede (Nagpal & Kanwar, 1981). The possibility that these granule phospholipid stores may also serve as the potential reservoirs for *de novo* membrane generation during the activation of their respective secretory systems should be investigated.

It should be pointed out that many of the newly assembled vesicles observed here (Figs 3–5), with diameters in the range of 23–25 nm, are similar in size to the unilamellar vesicles formed *in vitro* by exhaustive sonication of phospholipids in aqueous media (Huang, 1969; Sheetz & Chan, 1972). Small vesicles with diameters less than 40 nm have been shown to be highly unstable and fusogenic below their gel to liquid-crystalline phase transition temperature (Lichtenberg *et al.*, 1981). Their fusion is also enhanced by divalent cations such as calcium, and by the presence of an osmotic gradient across the vesicle membrane (Ohki, 1984). A similar condition conducive to membrane fusion is also believed to exist in the granule.

Due to limitation of the spatial arrangement of phospholipid molecules and geometrical constraints imposed on a minute sphere, there is a high degree of asymmetry between the outer and inner monolayers of a small vesicle. This is illustrated in Fig. 6 for a vesicle of 23 nm in diameter. Since the phospholipid molecules are assumed to be arranged in unit cells





**Fig. 5.** Granule matrix changes during new membrane assembly. (A) Vesicles (arrows) appear to be emerging from the granule matrix like soap bubbles following activation. Many vesicles also show electron-dense material within them. Vesicles (arrowhead) also appear to be able to emerge from deep within the granule matrix, suggesting a loosening of the matrix to permit water infiltration. Comparison of the perigranular membrane with the granule matrix suggests that the granule radius has increased by about 20%. This increment in radius corresponds to about 40% increase in the perigranular membrane surface area. (B) New vesicles (arrows) appear to have fused with each other. The loosening of the matrix as a result of water infiltration, and the sequestration and coalescence of hydrophobic elements, have caused the matrix to take on a dappled appearance. The bar at the lower left corner represents 0.1  $\mu\text{m}$ .

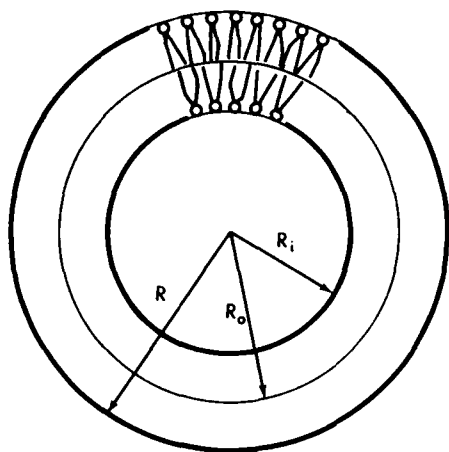


Fig. 6. Cross-section of a small vesicle showing asymmetric packing of phospholipid molecules between the inner and outer phospholipid monolayers. If the diameter of the vesicle is 23 nm, the vesicle radius,  $R$ , equals 11.5 nm. If the thickness of the bilayer is 5 nm, then  $R_o$  ( $= R - 2.5$  nm) = 9 nm and  $R_i$  ( $= (R_o - 2.5$  nm) = 6.5 nm.

contained within a cone with its apex situated in the centre of the vesicle, the maximum number of molecules that can be packed into the outer monolayer and inner monolayer of this vesicle would depend on the spherical surface areas defined by the radii,  $R_o$  and  $R_i$ , respectively (Huang & Mason, 1978). If we assume a value of 5 nm for the bilayer thickness (Small, 1967), for a vesicle with a diameter of 23 nm ( $R = 11.5$  nm), the outer limiting radius,  $R_o$ , would be equal to 9 nm (11.5 nm minus 2.5 nm); and the inner limiting radius,  $R_i$ , would be equal to 6.5 nm (9 minus 2.5 nm). If we assume that the vesicle contains only phospholipid molecules of equal dimension, an equation which defines the ratio of the number of molecules in the outer monolayer ( $n_o$ ) to that in the inner monolayer ( $n_i$ ) can be derived.

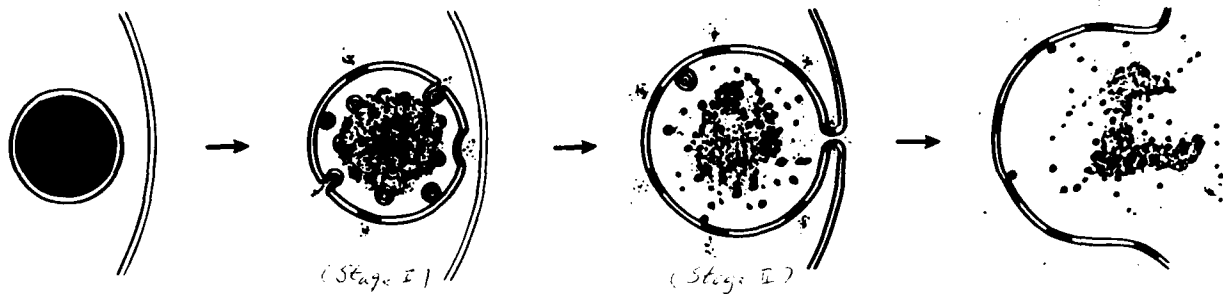
$$n_o/n_i = (R_o)^2/(R_i)^2 \quad \text{Equation (1)}$$

By substituting the values of 9 nm for  $R_o$  and 6.5 nm for  $R_i$  into equation (1), it can be shown that  $(n_o/n_i) = 1.9$ . This means that for a vesicle of 23 nm in diameter, there are almost twice as many phospholipid molecules in the outer monolayer as in the inner monolayer. A value of  $(n_o/n_i) = 2.1$  has been approximated for phospholipid vesicles of 21 nm in diameter using a method involving  $^{31}\text{P}$  NMR (Yeagle *et al.*, 1976). Since phospholipid molecules cannot readily diffuse from one monolayer into the opposite monolayer, a phenomenon known as 'flip-flop' (Rothman & Lenard, 1977), the fusion of a small vesicle into a planar bilayer may result in the temporary appearance of a small surface curvature or 'bulge' in the planar membrane. This phenomenon might have been observed in the images of perigranular membrane cross-sections (Fig. 4B and C), where the large number of membrane

curvatures or 'kinks' may reflect the insertion of many vesicles.

The biochemical mechanism of how cross-linking of mast cell surface receptor-bound IgE by its specific antigen can trigger the release of histamine, has been studied by T. and K. Ishizaka and their colleagues (T. Ishizaka, 1982; Kagey-Sobotka *et al.*, 1982; Metzger *et al.*, 1982; K. Ishizaka, 1985). However, it is still unclear how the receptor-mediated signal transduction can result in the activation of secretory granules throughout the cell. Since spontaneous sequestration and coalescence of phospholipid into the membrane bilayer requires the presence of water, we have proposed earlier that one of the results of signal transduction in the stimulus-secretion coupling process must be the initiation of water influx into the target secretory granules (Chock & Chock, 1985). At the onset of water influx, the matrix-bound phospholipid and other membrane components situated directly under the perigranular membrane are immediately sequestered into bilayer vesicles. Their fusion with the perigranular membrane results in rapid lifting and expansion of the perigranular membrane away from the granule matrix. Since secretory granules have never been seen to migrate rapidly within the filament-filled cytoplasm, or to undergo Brownian movement, a rapid enlargement of the perigranular membrane would provide the necessary mechanism by which an activated granule can achieve contact with the plasma membrane in order that fusion and pore formation can occur.

A graphic representation of such a process is summarized in Fig. 7. This simple scheme may explain how a dense quiescent granule, following activation to secrete histamine, can progressively enlarge prior to fusion with the plasma membrane. The process of new membrane assembly (Fig. 7, stage I) involves the spontaneous sequestration and coalescence of matrix-stored phospholipid and other membrane precursor elements into membrane vesicles. Rapid fusion of these *de novo* generated vesicles with the perigranular membrane results in enlargement of the perigranular membrane. Since this spontaneous membrane assembly process can also result in incorporation of matrix materials in the new vesicles, their fusion with the perigranular membrane will also result in extrusion of some granule matrix content into the cytoplasm (Schmauder-Chock & Chock, 1987b; Chock & Schmauder-Chock, 1989). In conjunction with this membrane assembly, the granule matrix begins to decondense and sometimes take on a dappled appearance, as shown in Fig. 5B. Subsequently, the expanding perigranular membrane comes into contact with the plasma membrane and their fusion results in pore formation (Fig. 7, stage II). If the expanding perigranular membrane comes into contact with neighbouring granules, their fusion will result in the formation of a



**Fig. 7.** A two-stage fusion model for exocytosis. The figures from left to right depict the progression of granule exocytosis. Activation of a dense, quiescent granule near the plasma membrane results in an influx of cellular water into the granule. The entry of water causes spontaneous sequestration and coalescence of the matrix-bound phospholipid and other membrane precursors into membrane vesicles (stage I). The influx of water also initiates the granule activation process. Rapid fusion of the newly assembled vesicles (represented by shaded bilayer) into the perigranular membrane results in the enlargement of the perigranular membrane. Since the *de novo* generated vesicles may contain some matrix materials, their fusion with the perigranular membrane can also result in the ejection of granule contents into the cytoplasm. When the expanding perigranular membrane comes into contact with the plasma membrane, their fusion results in pore formation (stage II). Following pore formation, the granule matrix can rapidly unravel and disperse.

membrane-lined common vacuole (Fig. 4), as in compound exocytosis. Fusion of this vacuole with the plasma membrane will result in extrusion of multiple granule matrices. This may be the mechanism by which granules situated deep within the cytoplasm can be exteriorized.

The process of spontaneous membrane assembly postulated here requires no chemical reaction. It is a mere physical phenomenon driven by free energy and entropy which is typical of the interaction between amphipathic molecules and water (Danielli & Davson, 1935; Frank & Evans, 1945; Tanford, 1980). The spontaneity and rapidity of such a process is reflected in the ease with which bilayer membrane can be made *in vitro* (Langmuir & Waugh, 1938; Mueller *et al.*, 1962; Andreoli, 1974). The involvement of an entropy-driven spontaneous process in the mechanism of exocytosis would explain why secretion can occur within seconds of activation (Douglas, 1974; Plattner *et al.*, 1984). The varying sizes and shapes of the new membranes, as seen here for the activated mast cell granules, not only reflect the fluid character of the biological membrane but also the randomness of the spontaneous process.

### Conclusion

By invoking the normal physiological cascade involved in the triggering of the anaphylactic reaction, we have observed evidence which strongly suggests the occurrence of a *de novo* membrane assembly process in the mechanism of mast cell granule exocytosis. Mobilization of this process, following antigen challenge of presensitized mast cells, enables the perigranular membrane of the activated granule to enlarge and fuse with the plasma membrane, forming a pore through which histamine and other mediators of inflammation can be exteriorized. This finding also

explains why activated granules of stimulated mast cells have been reported to undergo 'swelling' (enlargement), and to contain extra membrane in conjunction with histamine release. This observation, together with those reported in mast cells stimulated by a detergent and by compound A23187, supports our hypothesis that a spontaneous process of new membrane assembly occurs in the mechanism of secretory granule exocytosis. Since the site of new membrane assembly is in the secretory granule where preformed membrane precursors are stored (Chock & Schmauder-Chock, 1989), it seems that this process involves a mechanism different from that believed to take place on the smooth endoplasmic reticulum where phospholipid synthesis is coupled to the process of membrane assembly (Wilgram & Kennedy, 1963; Alberts *et al.*, 1983). The underlying mechanism of a granule exocytosis-initiated membrane assembly might involve a combination of spontaneous free energy and entropy changes associated with the mixing of amphipathic molecules with water. The important role that water plays in this membrane assembly process cannot be over-emphasized in view of the fact that most, if not all, biological processes (including enzyme reactions, protein synthesis, nucleic acids synthesis, lipid synthesis, as well as the assembly and disassembly of biologically macromolecules) require the participation of water molecules at some point or other; furthermore, such processes take place in a mostly aqueous cellular medium.

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